



Published in final edited form as:

Sex Transm Dis. 2018 January ; 45(1): 61–68. doi:10.1097/OLQ.0000000000000694.

The Etiology of Genital Ulcer Disease and Coinfections With *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Zimbabwe: Results From the Zimbabwe STI Etiology Study

More Mungati, MBChB, MPH^{*}, Anna Machiha, DNS^{*}, Owen Mugurungi, MD^{*}, Mufuta Tshimanga, MD, MPH[†], Peter H. Kilmarx, MD^{‡,§}, Justice Nyakura, MPH^{*}, Gerald Shambira, MD, MPH[†], Vitalis Kupara, DNS[¶], David A. Lewis, FRCP (UK), PhD^{||,**}, Elizabeth Gonese, MPH[‡], Beth A. Tippet Barr, DrPH[‡], H. Hunter Handsfield, MD^{††}, and Cornelis A. Rietmeijer, MD, PhD, MSPH^{‡‡,§§}

^{*}Zimbabwe Ministry of Health and Child Care

[†]Department of Community Medicine, University of Zimbabwe, College of Health Sciences, Harare, Zimbabwe

[‡]US Centers for Disease Control and Prevention, Zimbabwe and Division of Global HIV/AIDS, CDC, Atlanta, GA

[§]Fogarty International Center, National Institutes of Health, Bethesda, MD

[¶]Zimbabwe Community Health Intervention Research (ZiCHIRe) Project, Harare, Zimbabwe

^{||}Western Sydney Sexual Health Centre, Parramatta

^{**}Marie Bashir Institute for Infectious Diseases and Biosecurity & Sydney Medical School—Westmead, University of Sydney, Sydney, New South Wales, Australia

^{††}School of Medicine, University of Washington, Seattle, WA

^{‡‡}Colorado School of Public Health, University of Colorado Denver, Denver, CO

^{§§}Rietmeijer Consulting LLC, Denver, CO

Abstract

Background—In many countries, sexually transmitted infections (STIs) are treated syndromically. Thus, patients diagnosed as having genital ulcer disease (GUD) in Zimbabwe receive a combination of antimicrobials to treat syphilis, chancroid, lymphogranuloma venereum (LGV), and genital herpes. Periodic studies are necessary to assess the current etiology of GUD and assure the appropriateness of current treatment guidelines.

Materials and Methods—We selected 6 geographically diverse clinics in Zimbabwe serving high numbers of STI cases to enroll men and women with STI syndromes, including GUD.

Correspondence: Cornelis A. Rietmeijer, MD, PhD, MSPH, Rietmeijer Consulting, LLC 533. Marion Street, Denver CO 80218. kees@rietmeijer.us.

Disclaimer

The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Sexually transmitted infection history and risk behavioral data were collected by questionnaire and uploaded to a Web-based database. Ulcer specimens were obtained for testing using a validated multiplex polymerase chain reaction (M-PCR) assay for *Treponema pallidum* (*TP*, primary syphilis), *Haemophilus ducreyi* (chancroid), LGV-associated strains of *Chlamydia trachomatis*, and herpes simplex virus (HSV) types 1 and 2. Blood samples were collected for testing with HIV, treponemal, and nontreponemal serologic assays.

Results—Among 200 GUD patients, 77 (38.5%) were positive for HSV, 32 (16%) were positive for *TP*, and 2 (1%) were positive for LGV-associated strains of *C trachomatis*. No *H ducreyi* infections were detected. No organism was found in 98 (49.5%) of participants. The overall HIV positivity rate was 52.2% for all GUD patients, with higher rates among women compared with men (59.8% vs 45.2%, $P < 0.05$) and among patients with HSV (68.6% vs 41.8%, $P < 0.0001$). Among patients with GUD, 54 (27.3%) had gonorrhea and/or chlamydia infection. However, in this latter group, 66.7% of women and 70.0% of men did not have abnormal vaginal or urethral discharge on examination.

Conclusions—Herpes simplex virus is the most common cause of GUD in our survey, followed by *T. pallidum*. No cases of chancroid were detected. The association of HIV infections with HSV suggests high risk for cotransmission; however, some HSV ulcerations may be due to HSV reactivation among immunocompromised patients. The overall prevalence of gonorrhea and chlamydia was high among patients with GUD and most of them did not meet the criteria for concomitant syndromic management covering these infections.

Sexually transmitted infections (STIs) associated with genital ulcer disease (GUD) continue to be an important cause of morbidity and mortality worldwide. Although the etiology of GUD varies in different parts of the world, herpes simplex virus (HSV) is considered the leading cause of this syndrome globally. In a recently published study,¹ the World Health Organization estimates that in 2012, 19.2 million people aged 15–49 years were newly infected with HSV type 2 (HSV-2) resulting in 417 million people in this age group living with HSV-2 infection, and a global prevalence of 11.3%. Adding an increasing number of genital herpes caused by herpes simplex type 1 virus (HSV-1), it is estimated that more than 40 to 80 million people suffer from recurrent genital herpes in any given year. In addition to this morbidity, HSV-2 infection is an important risk factor for HIV acquisition^{2,3} and can cause debilitating disease in the maternally exposed fetus and neonate.^{4,5} The global HSV burden disproportionally affects Africa with more than 30% of people aged 15 to 49 years chronically infected.¹

Likewise, Africa and specifically sub-Saharan Africa are also disproportionally affected by syphilis. Although the incidence of syphilis is substantially lower than genital herpes and has been declining over recent years, it is estimated that in 2012, 5.6 million incident syphilis cases occurred worldwide of which 32% in sub-Saharan Africa.⁶ Syphilis continues to be a major global public health problem. In 2012, an estimated 930,000 maternal syphilis infections caused 350,000 adverse pregnancy outcomes, including 143,000 early fetal deaths and stillbirths, 62,000 neonatal deaths, 44,000 preterm or low weight births, and 102,000 infected infants worldwide.⁷

The relative proportion of GUD caused by chancroid seems to be decreasing. For example, in a study from Botswana in 2002, 2% of patients with GUD were infected with *Treponema pallidum* and 1% with *Haemophilus ducreyi*, compared to 58% with HSV-2.⁸ In a study from Malawi conducted between 2004 and 2006, the proportion of GUD caused by *H ducreyi* was higher (15%) compared with *T pallidum* and lymphogranuloma venereum (LGV) strains of *Chlamydia trachomatis* (both at 6%), whereas 67% tested positive for HSV-2.⁹ In a more recent study from Zambia, however, none of the patients with GUD had *H ducreyi* detected, whereas 3% were positive for LGV strains of *C trachomatis*.¹⁰ Changes in the underlying epidemiology of GUD have been linked to the change in management when the World Health Organization introduced syndromic management for the treatment of GUD after 2000.¹¹

In the absence of etiologic testing, STI reporting and management in many countries, including Zimbabwe, is based on presenting symptoms. Thus, in 2015, a total of 252,406 adult STI cases were reported syndromically to the Zimbabwe Ministry of Health and Child Care, including 20,063 men and 18,996 women with GUD, in addition to 56,540 men with urethral discharge, 79,371 women with vaginal discharge, 27,782 women with pelvic inflammatory disease, and 21,489 men and 26,165 women with other STI syndromes, including genital warts (Zimbabwe Ministry of Health and Child Care, unpublished data, 2015).

Syndromic treatment of GUD, as recommended by the Zimbabwe Ministry of Health and Child Care, includes the combined use of benzathine penicillin, erythromycin, and acyclovir to treat potential infections with *T pallidum*, *H ducreyi*, LGV-associated *C trachomatis* strains, and HSV, respectively.¹² However, no studies have been undertaken in Zimbabwe in recent years to determine the underlying etiology of GUD and thus assess the adequacy of current treatment guidelines.

As part of a recently completed STI etiology study in Zimbabwe, the primary aim of this analysis was to determine the presence of syphilis, chancroid, LGV, and genital herpes infections among women and men presenting with GUD. Secondary aims were to determine the presence of vaginal or urethral *Neisseria gonorrhoeae* and *C trachomatis* as well as the presence and association of HIV infections with GUD in this population.

MATERIALS AND METHODS

The Zimbabwe STI Etiology Study was conducted in 2014 and 2015 to determine the causation of the most important STI-associated syndromes in Zimbabwe: male urethral discharge syndrome, vaginal discharge syndrome, and GUD. A full description of study methods is available online.¹³ The most relevant details are summarized below. Results of the female and male genital discharge syndromes will be reported in separate articles.

Clinic Selection

Using 2012 surveillance statistics, we identified a regionally diverse sample of 6 clinics with high numbers of reported STI syndromes. Specifically, we selected 2 clinics from Harare (Mbare and Budiro), the country's capital and largest city in the northeastern part of the

country; 2 clinics in Bulawayo (Nkulumane and Khami Road), the second largest city located southwest and ethnically distinct from Harare; 1 clinic from Beitbridge (Dulibadzimu) on the southern border with South Africa; and finally, 1 clinic from rural Gutu (Gutu Road Hospital), near the country center. These clinics were deemed to be sufficiently representative of risk populations regionally to yield meaningful results. More details on clinic selection can be found elsewhere.¹³

For purposes of the analysis in this study, clinics were combined into 3 regions: Harare, Bulawayo, and Beitbridge/Gutu.

Sample Size

We aimed to enroll 200 participants in each of the STI syndromes categories, including 100 men and 100 women presenting with GUD. This sample size was deemed to yield sufficiently robust positivity estimates of the major GUD causes and at the same time be practical from the perspective of the project's limited budget and enrollment window. Because the study population was, in essence, a convenience sample, we did not intend to apply sample weights.¹³

Patient Selection

During the enrolment period at each clinic, all sexually active women and men aged 18 to 55 years presenting with GUD were eligible for the study. Excluded from the study were those who did not speak English, Shona, or Ndebele (the 3 major languages in Zimbabwe); those unable to provide consent; those who had received antibiotics for STI treatment in the previous 4 weeks; or those previously enrolled in the study.

Study Procedures

A team of 3 nurses was trained in study procedures and deployed sequentially for a period of 10 to 17 weeks to each of the 6 study sites, starting in the Harare clinics, then moving to Bulawayo and finally to Beitbridge and Gutu. The start of enrollment at each site was preceded by at least 2 site visits involving the study leadership including the team lead (V.K.), the lead consultant (C.A.R.), and senior researchers representing the Zimbabwe Ministry of Health and Child Care (A.M. and M.M.).

Patients with GUD were enrolled after obtaining informed consent and a paper-copy questionnaire was completed by the study nurse that included demographic information, sexual history, description of symptoms, history of STI/HIV, and current use of medications. Patients with GUD had swabs taken from the ulcer bases. Vaginal swabs for women and urine samples for men were obtained for chlamydia and gonorrhea testing. Blood specimens were collected from patients for syphilis serology and HIV testing. A separate consent for HIV testing was obtained. Refusing a blood draw was not a reason for exclusion from the study.

All patients were treated for their presenting STI syndrome according to the 2013 Zimbabwe STI treatment guidelines.¹² After completion of the visit, paper data were reviewed by the

lead study nurse and then transcribed into a computer-based data system on handheld devices. Data were uploaded daily from study sites to an online secure central database.

Laboratory procedures

All specimens were kept refrigerated after collection and shipped in a cooler box with cooling packs by courier overnight to the receiving laboratory at Wilkins Hospital in Harare, where all samples were kept refrigerated until further processing.

A number of tests were conducted at the receiving laboratory, including rapid HIV serologic testing using the standard testing algorithm in Zimbabwe (HIV testing in Zimbabwe follows a standard algorithm of the following HIV rapid tests: 1) initial test by First Response HIV 1-2.O [Premier Medical Corporation, Daman, India]; 2) confirmatory test by Alere Determine HIV 1/2 [Alere, Waltham MA] if the initial test result is positive, and 3) INSTI HIV1/HIV2 [Biolytical, Richmond BC, Canada] as a tiebreaker if the initial and confirmatory tests are discrepant), treponemal testing by SD Bioline DUO rapid test (Standard Diagnostics Inc, Gyeonggido, Republic of Korea), and *T pallidum* haemagglutination assay (TPHA, SPINREACT, Girona, Spain), and nontreponemal testing by rapid plasma reagin (RPR, SPINREACT, Girona, Spain). These tests were all conducted according to test package inserts.

All ulcer samples were stored in a -70°F freezer and batched for shipment to the STI reference laboratory at the National Institute of Communicable Diseases (NICD) in Johannesburg, South Africa. The multiplex polymerase chain reaction (M-PCR), used to determine ulcer etiology in this study, is a modified version of the real-time M-PCR assay acquired by the NICD through technology transfer from the US Centers for Disease Control and Prevention (CDC).¹⁴ The original CDC assay, developed by Roche Molecular systems, detected *T pallidum*, *H ducreyi*, and HSV with a sensitivity of at least 10 copies for each organism when all 3 targets were present.¹⁵ At the NICD, this real-time assay was subsequently modified into a quadriplex assay that additionally detected *C trachomatis*. DNA extracts from positive *C trachomatis* specimens were subsequently tested with an in-house LGV-specific real-time PCR assay,¹⁶ and DNA extracts from HSV specimens were subsequently typed using a commercial HSV-1/HSV-2 assay (Sacace Biotechnologies Srl, Como, Italy). All 3 assays were validated within NICD using known positive and negative specimens (unpublished data), and extracted genomic DNA specimens were used as controls for all 4 ulcer pathogens.

Vaginal and urine samples were shipped to 2 local laboratories and tested for *N gonorrhoeae* and *C trachomatis* by nucleic acid amplification testing (NAAT) using Becton Dickinson ProbeTec (BD Molecular Diagnostics, Franklin Lakes, NJ) at the University of Zimbabwe/University of California San Francisco laboratory in the Obstetrics and Gynaecology Department of the University of Zimbabwe School of Medicine and using GeneXpert (Cepheid, Sunnyvale, CA) at the Flowcytometry laboratory in Harare. All tests were done according to test package inserts under standard operating procedures. All urine and vaginal specimens were tested on both platforms with greater than 95% concordance of test results.¹⁷ For the purposes of this study, we considered a chlamydia or gonorrhea test result to be

positive when it was positive on either platform. More detail on laboratory procedures can be found in the online supplement.¹³

Statistical Methods

Data on participant demographics, sexual health, and STI history were analyzed using SAS software (Cary, NC). Tests for statistical significance included the χ^2 test for categorical variables and Student *t* test for continuous variables. Because we assumed the possibility of variations and clustering by clinic, we did not attempt to generalize the relative prevalence of pathogens associated with GUD in our study and hence do not present 95% confidence intervals.

Institutional Review

The protocol, including consent forms and questionnaires, was reviewed and approved by the Joint Research and Ethics Committee of Parirenyatwa Central Hospital, the Zimbabwe Medical Research Council, and the US CDC.

RESULTS

Participant Recruitment

Of the 100 women and 100 men with GUD, M-PCR results were available for all ulcer specimens; however, urine samples taken from 2 men were inadequate for gonorrhea/chlamydia NAAT. Enrollment data by study site are summarized in Table 1. Only 4 women and 1 man were recruited at Gutu Road Hospital. The low enrollment at the Gutu site was the result of low STI patient volume and a pragmatic decision to focus our limited resources on sites where patient volume was higher and recruitment more productive. Nonetheless, we did not see any reason to exclude these persons from our analyses. For statistical purposes, we included them with the Beitbridge sample.

Demographic Characteristics

As expected, a higher proportion of men and women enrolled at the Bulawayo clinics reported Ndebele ethnicity, because this is the capital of Matabeleland with a prominent Ndebele population. In addition, there was a statistically significant difference across regions in the number of participants who reported more than 1 sex partner in the previous 3 months, with participants from Beitbridge/Gutu reporting the highest number of partners ($P < 0.05$). There were no significant differences between regions with regard to age, condom use with main or nonmain partners, self-reported HIV status, and a history of STI (Table 1).

Men in the study were significantly older (mean, 29.6 years; median, 28.5 years) than women (mean, 27.9 years; median, 26 years; $P < 0.05$), and men were also significantly more likely to be unmarried (68% vs 51%, $P < 0.05$) and less likely to be unemployed when compared with women (59% vs 89%, $P < 0.001$). However, there were no differences between men and women with respect to number of sex partners in the past 3 months, condom use with main or nonmain partners, self-perceived HIV status, or history of STI (data not shown).

Etiology of GUD

Overall, 77 (38.5%) of 200 patients with GUD were infected with HSV (all but one typed as HSV-2), and 32 (16%) of 200 had *T pallidum* infection detected by M-PCR. Nine patients (4.5%) were dually infected with HSV and *T pallidum*. Only 2 patients (1%) had *C trachomatis*-LGV strains detected, and none had *H ducreyi* infection identified. No organism was found in 49.5% of participants (Table 2). Of those with any pathogen detected ($n = 101$), 76.2% had HSV, 31.7% had *T pallidum* infection, and 1.9% had chlamydia-LGV strains. Herpes simplex virus infection was the most common cause of GUD in both women (39%) and men (38%), followed by *T pallidum* infection (13% of women and 19% of men). There was no statistically significant difference between men and women with respect to causative organisms ($P = 0.88$).

Multiplex PCR results varied by study site, with Harare sites having significantly higher proportions of patients with no pathogen identified (65.3% for Harare sites vs 37.8% for Bulawayo sites vs 44.4% for Beitbridge, $P < 0.05$). However, the proportion of patients diagnosed as having HSV and treponemal infections were not different across sites when excluding patients who had no pathogens identified (data not shown).

In a further analysis of patients either infected with HSV or *T pallidum* (thus excluding those with mixed infections), we found that shorter duration of symptoms (1–7 vs >7 days) was significantly associated with detection of HSV, but not *T pallidum*. Significantly lower rates of HSV were found in patients concomitantly infected with *C trachomatis*. Rates were also lower among patients infected with *N gonorrhoeae*, but this difference was not statistically significant. Finally, higher HSV rates were also observed among patients who self-reported as HIV infected (Table 3).

Treponemal and Nontreponemal Test Results

Blood specimens from 181 patients were available (19 patients declined to submit a blood sample). Valid RPR results were available for all 181 patients, of which 37 (20.4%) were positive, and valid TPHA results for 165 patients, of which 42 (25.4%) were positive. Both RPR and TPHA were positive for 26 (15.8%) of 165 patients: 15 (55.5%) of 27 testing positive for *T pallidum* and 11 (7.9%) of 138 testing negative (Table 4).

HIV Coinfection

HIV test results were available for 180 of 181 patients who submitted a blood sample. Overall, 59.8% of women and 45.2% of men with GUD tested positive for HIV ($P < 0.05$). There was no variation by region. Testing results for the causative agent by M-PCR varied significantly by HIV status. Specifically, patients with HSV had higher rates of HIV infection (68.6%) compared with patients without HSV (41.8%, $P < 0.0001$). Conversely, when excluding mixed infections ($n = 170$), HIV-positive participants had a higher positivity rate of HSV infection compared with HIV-negative participants (53.9% vs 28.4%, $P < 0.0001$), but similar positivity of *T pallidum* (20.5% vs 17.2%, Table 3), with no sex differences observed (data not shown).

Chlamydia and Gonorrhea Infection

Results of genital *C trachomatis* and *N gonorrhoeae* testing are summarized in Table 5. Overall, 31 (31.0%) of 100 women and 23 (23.5%) of 98 men were NAAT positive for either infection. After excluding those with missing observations, 20 (66.7%) of 30 women and 14 (70.0%) of 20 men with either infection had no vaginal or urethral discharge observed. Thus, of 54 men and women with GUD and concurrent chlamydia and/or gonorrhea infection, 34 (63.0%) were not eligible for concomitant genital discharge syndrome management, representing 17% of all patients with GUD in our study.

DISCUSSION

Our study results confirm the continued high prevalence of HSV infections as the probable cause of GUD. However, HSV positivity in our study (38.5%) was lower than that found in recent surveys in the southern African region: 60.5% in Botswana (2002),⁸ 62% in Mozambique (2005),¹⁸ 67% in Malawi (2004–2006),⁹ and 73.6% in South Africa (2005–2006),¹⁴ but higher than a more contemporary study in Zambia, Zimbabwe's neighbor to the north: 28% (2010).¹⁰ A common finding among these and our studies is the significant association between HSV and HIV infection; close to 54% of persons with HSV in our study were HIV infected, compared to 28% without HSV. In our study, we could not distinguish primary HSV infections from recurrences, but in the Malawi study, 75% of HSV infections were recurrences.⁹ Thus, the prevalence of HSV infection among persons presenting with GUD seems to be influenced by recurrent HSV infection among HIV-infected patients, especially those experiencing immunosuppression. Because access to HIV antiretroviral therapy is rapidly increasing in many sub-Saharan countries, including Zimbabwe, the epidemiology and relative prevalence of etiologic determinants of GUD may change.

Although HSV infections may be declining, the relative prevalence of syphilis as a cause of GUD may be increasing. In our study, 16% of patients with GUD tested positive for *T pallidum* and an additional 8% of patients submitting blood specimens had negative *T pallidum* results but positive RPR and TPHA test results suggesting recent syphilis. Thus, overall, up to 24% of patients with GUD in our study had evidence of active syphilis.

T pallidum rates in our study were considerably higher than rates found in the Botswana (5.1%),⁸ Mozambique (0%),¹⁸ Malawi (6%),⁹ and South African (4.9%)¹⁴ studies. However, these studies were conducted 10 to 14 years ago, a time frame that saw rapid increases in early syphilis cases in the United States and Europe. Although the recent epidemiology of syphilis in the United States and Europe is largely driven by syphilis increases among men who have sex with men, there are now indications that syphilis is also increasing among heterosexuals with subsequent rises in congenital syphilis rates,¹⁹ and there is no reason to assume that the epidemiology in other world regions, including Africa, might be different. Indeed, the more recent study from Zambia also saw higher *T pallidum* rates (10%).¹⁰ Of course, a study of a highly selective group of patients presenting with STI cannot be generalized and, as discussed earlier, the relative proportion of syphilis cases may also be a function of a decline in genital herpes cases in our study. Nonetheless, our study raises the possibility of reemerging syphilis in Zimbabwe and the southern African region that should

be further studied and, in the meantime, should reinforce efforts to screen pregnant women to prevent congenital syphilis.

Our study confirms the decline of chancroid as a cause of GUD as shown previously in other African and non-African countries^{8,9} the most recent study from Zambia failed to detect any *H ducreyi* infections.¹⁰ In 1979, chancroid still accounted for 38% of STI cases in Zimbabwe's capital Salisbury (now Harare) but declined to just 3% among men with GUD in 1995,²⁰ and in the current study, no cases were found. This decrease may be due to either syndromic management used in treating STIs or widespread use of antibiotics treating other infections. The apparently sustained decline in chancroid in Zimbabwe and the southern African region has called into question whether syndromic treatment guidelines should continue to make specific provisions for treating this disease.¹⁰

Compared with previous studies in the region, we found a higher proportion of GUD cases with unknown etiology, that is, 49.5% compared with 39.4% in Botswana,⁸ 30% in Mozambique,¹⁸ 20% in Malawi,⁹ and 20.6% in South Africa.¹⁴ By contrast, the more recent study in Zambia found a higher proportion: 55%.¹⁰ We found significantly lower rates of any pathogens detected in the Harare clinics, and because these were the first clinics to enroll patients, we cannot rule out that staff training, differences in patient selection, and quality of ulcer sampling could have played a role. As has been shown in other studies, retrieving etiologic agents, especially HSV, is dependent on how long lesions have been present.¹⁰ In our study, we confirmed that significantly fewer cases of HSV infection were found in persons with lesions 7 days or older. No such association was found for *T pallidum*.

Finally, we documented a high rate of chlamydia and gonorrhea comorbidity among our patients with GUD, with 31% of women and 23.5% of men being coinfecting. Further analysis indicated that 63% of women and men with coinfections did not have vaginal or urethral discharge and would thus not receive optimal treatment for these infections. These represent 17% of all patients with GUD in this study. This finding suggests that current syndromic management guidelines may be inadequate for women and men with GUD with concurrent gonorrhea and chlamydia. Other studies in the region have yielded similar results suggesting this comorbidity be addressed in future revision of the guidelines.¹⁴

The findings of our study are subject to a number of limitations. First, we included clinics that also provide care to HIV-infected people. Thus, we may have oversampled HIV-infected people for this study and, because HIV infection was closely related to HSV infections, may have caused a bias in study results. Second, the variation in etiologic patterns by clinic location suggests potential limitations in generalizability. Third, as has already been mentioned, the relatively large proportion of GUD patients with no pathogens identified may indicate biases in patient selection and consistency of study and testing procedures. Finally, by its nature, only symptomatic patients were enrolled in the study, and generalizations outside this group to the general population cannot be made.

In conclusion, HSV-2 infection was the most common cause of GUD in our study, followed by syphilis. No cases of chancroid were found. The strong association of concurrent HIV and HSV infections may in part be explained by recurrent genital herpes in

immunocompromised patients. Nonetheless, the high co-occurrence of HIV infection in this group of patients suggests very high risks of HIV transmission. Patients with GUD should therefore receive the highest priority for HIV prevention.

Acknowledgments

The authors thank the ZiCHIRE study team: Luanne Rodgers, Laboratory Scientist; State Registered Nurse (SRN); Mebbina Muswera, SRN and State Registered Midwife (SRM); Sarah Vundhla, SRN and SRM; and Shirley Tshimanga, SRN and SRM. This study could not have been conducted without the gracious support and collaboration of the staff and patients of the following clinics: Mbare and Budiriro clinics (Harare), Nkulumane and Khami Road clinics (Bulawayo), Dulibadzimu clinic (Beitbridge), and Gutu Rural Hospital (Gutu).

Funding

The Zimbabwe STI Etiology Study was supported by funds from the President's Emergency Plan for AIDS Relief through a cooperative agreement between the US Centers for Disease Control and Prevention and the University of Zimbabwe Department of Community Medicine SEAM Project under the terms of Cooperative Agreement Number IU2GGH000315-01.

References

1. Looker KJ, Magaret AS, May MT, et al. Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012. *PLoS One*. 2015; 10:e0140765. [PubMed: 26510007]
2. Freeman EE, Weiss HA, Glynn JR, et al. Herpes simplex virus 2 infection increases HIV acquisition in men and women: Systematic review and meta-analysis of longitudinal studies. *AIDS*. 2006; 20:73–83. [PubMed: 16327322]
3. Chen CY, Ballard RC, Beck-Sague CM, et al. Human immunodeficiency virus infection and genital ulcer disease in South Africa: The herpetic connection. *Sex Transm Dis*. 2000; 27:21–29. [PubMed: 10654864]
4. Whitley RJ. Neonatal herpes simplex virus infections. *J Med Virol*. 1993; (Suppl 1):13–21. [PubMed: 8245879]
5. Kimberlin DW, Whitley RJ. Neonatal herpes: what have we learned. *Semin Pediatr Infect Dis*. 2005; 16:7–16. [PubMed: 15685144]
6. Newman L, Rowley J, Vander Hoorn S, et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. *PLoS One*. 2015; 10:e0143304. [PubMed: 26646541]
7. Wijesooriya NS, Rochat RW, Kamb ML, et al. Global burden of maternal and congenital syphilis in 2008 and 2012: A health systems modelling study. *Lancet Glob Health*. 2016; 4:e525–e533. [PubMed: 27443780]
8. Paz-Bailey G, Rahman M, Chen C, et al. Changes in the etiology of sexually transmitted diseases in Botswana between 1993 and 2002: Implications for the clinical management of genital ulcer disease. *Clin Infect Dis*. 2005; 41:1304–1312. [PubMed: 16206106]
9. Phiri S, Zadrozny S, Weiss HA, et al. Etiology of genital ulcer disease and association with HIV infection in Malawi. *Sex Transm Dis*. 2013; 40:923–928. [PubMed: 24220352]
10. Makasa M, Buve A, Sandøy IF. Etiologic pattern of genital ulcers in Lusaka, Zambia: Has chancroid been eliminated? *Sex Transm Dis*. 2012; 39:787–791. [PubMed: 23001266]
11. González-Beiras C, Marks M, Chen CY, et al. Epidemiology of *Haemophilus ducreyi* Infections. *Emerg Infect Dis*. 2016; 22:1–8. [PubMed: 26694983]
12. Zimbabwe Ministry of Health and Child Welfare. Management of Sexually Transmitted Infections and Reproductive Tract Infections in Zimbabwe. 2012. Available at: http://nac.org.zw/sites/default/files/STI%20Guidelines%20MoHCW%20Final%20Draft_05Dec2012.pdf
13. The Zimbabwe STI Aetiology Study Group. The Aetiology of Sexually Transmitted Infections in Zimbabwe—Study Protocol. 2014. Available at: <http://www.stdpreventiononline.org/index.php/resources/detail/2039>

14. Lewis DA, Müller E, Steele L, et al. Prevalence and associations of genital ulcer and urethral pathogens in men presenting with genital ulcer syndrome to primary health care clinics in South Africa. *Sex Transm Dis.* 2012; 39:880–885. [PubMed: 23064538]
15. Orle KA, Gates CA, Martin DH, et al. Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. *J Clin Microbiol.* 1996; 34:49–54. [PubMed: 8748271]
16. Morré SA, Spaargaren J, Fennema JS, et al. Real-time polymerase chain reaction to diagnose lymphogranuloma venereum. *Emerg Infect Dis.* 2005; 11:1311–1312. [PubMed: 16110579]
17. Mungati, M., Mugurungi, O., Machiha, A., et al. Performance of GeneXpert® CT/NG in the diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* among men and women with genital discharge syndrome in Zimbabwe. World STI and HIV Congress; Brisbane Australia. 2015. Available at: https://www.eiseverywhere.com/file_uploads/2bf18fa876f43162bf3cb3f2bf7cd3aa_KeesReitmeijer_P09.21.pdf. Accessed June 19, 2017
18. Zimba TF, Apalata T, Sturm WA, et al. Aetiology of sexually transmitted infections in Maputo, Mozambique. *J Infect Dev Ctries.* 2011; 5:41–47. [PubMed: 21330739]
19. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2015. Atlanta: U.S. Department of Health and Human Services; 2016.
20. O'Farrell N. Targeted interventions required against genital ulcers in African countries worst affected by HIV infection. *Bull World Health Organ.* 2001; 79:569–577. [PubMed: 11436480]

TABLE 1
Demographic and Behavioral Variables Among Patients Presenting With Genital Ulcer Disease in Zimbabwe

	Clinical Sites (by Region)							<i>P</i>
	Harare	%	Bulawayo	%	Beitbridge/Gutu	%	Total	
Total	72	36	74	37	54	27	200	
Sex								NS
Female	33	45.8	36	48.6	31	57.4	100	50.0
Male	39	54.2	38	51.4	23	42.6	100	50.0
Ethnicity								<0.0001
Shona	46	63.9	22	29.7	38	70.4	106	53.0
Ndebele	13	18.1	39	52.7	5	9.3	57	28.5
Other	13	18.1	13	17.6	11	20.4	37	18.5
Age, y								
Mean	29.1		28.2		28.4			
Median	28		27		27			
Age category, y								NS
15-19	6	8.3	5	6.8	2	3.7	13	6.5
20-24	16	22.2	24	32.4	14	25.9	54	27.0
25-29	20	27.8	16	21.6	14	25.9	50	25.0
30-34	15	20.8	17	23	14	25.9	46	23.0
35-39	6	8.3	6	8.1	8	14.8	20	10.0
40-44	4	5.6	4	5.4	1	1.9	9	4.5
45	5	6.9	2	2.7	1	1.9	8	4.0
Marital status								NS
Married	34	47.2	32	43.2	15	27.8	81	40.5
Unmarried	38	52.8	42	56.8	39	72.2	119	59.5
Employment								NS
Employed	22	30.6	19	25.7	13	24.1	54	27.0
Unemployed	50	69.4	55	74.3	41	75.9	146	73.0
No. partners in the previous 3 mo								
Mean	1.4		2		3			

	Clinical Sites (by Region)							P
	Harare	%	Bulawayo	%	Beitbridge/Gutu	%	Total	
Median	1		1		1			
Range	0-35		0-70		0-50			
>1 partner in the previous 3 mo								<0.05
No	64	88.9	54	73	38	70.4	156	78.0
Yes	8	11.1	20	27	16	29.6	44	22.0
Commercial sex in the previous 3 mo								NS
No	56	77.8	61	82.4	50	92.6	167	83.5
Yes	16	22.2	13	17.6	4	7.4	33	16.5
Condom use with the last sex main partner								NS
No	51	73.9	48	67.6	39	72.2	138	71.1
Yes	18	26.1	23	32.4	15	27.8	56	28.9
Not applicable	3		3				6	
Condom use with the last sex casual partner								NS
No	36	57.1	41	57.7	31	57.4	108	57.4
Yes	27	42.9	30	42.3	23	42.6	80	42.6
Not applicable	9		3				12	
Perceived HIV status								NS
Negative	20	27.8	14	18.9	9	16.7	43	21.5
Positive	22	30.6	33	44.6	25	46.3	80	40.0
Unknown	30	41.7	27	36.5	20	37	77	38.5
STI history								NS
No	44	65.7	38	52.1	29	53.7	111	57.2
Yes	23	34.3	35	47.9	25	46.3	83	42.8
Missing	5		1				6	

Percentages and χ^2 analysis limited to nonmissing data.

Some columns do not add up to 100% due to rounding.

NS indicates not significant; STI, sexually transmitted infection.

TABLE 2

Etiology of Genital Ulcer Disease in Zimbabwe

Pathogen	Clinical Sites (by Region)					
	Harare		Bulawayo		Beitbridge/Gutu	
	n	%	n	%	n	%
Total	72		74		54	
HSV	17	23.6	35	47.3	25	46.3
<i>T pallidum</i>	9	12.5	13	17.6	10	18.5
<i>C trachomatis</i>	1	1.4	0	0	1	1.9
<i>H ducreyi</i>	0	0	0	0	0	0
None	47	65.3	28	37.8	24	44.4
					99	49.5
						<0.01

NS indicates not significant; STD, sexually transmitted disease.

TABLE 3

Factors Associated with HSV and *T pallidum* Infection Among Patients With Genital Ulcer Disease in Zimbabwe*

	HSV				<i>T pallidum</i>			
	n	%	Positive	Negative	n	%	Positive	Negative
Total	68		99		22		99	
Sex								
Female	33	38.4	53	61.6	6	10.2	53	89.8
Male	35	43.2	46	56.8	16	25.8	46	74.2
Age, y								
15-19	3	33.3	6	66.7	1	14.3	6	85.7
20-24	18	43.9	23	56.1	10	30.3	23	69.7
25-29	15	34.9	28	65.1	4	12.5	28	87.5
30-34	17	42.5	23	57.5	6	20.7	23	79.3
35-39	6	35.3	11	64.7	1	8.3	11	91.7
40-44	4	44.4	5	55.6	0	0.0	5	100.0
45	5	62.5	3	37.5	0	0.0	3	100.0
Region								
Harare	15	24.2	47	75.8	7	13.0	47	87.0
Bulawayo	33	54.1	28	45.9	11	28.2	28	71.8
Beitbridge/Gutu	20	45.5	24	54.5	4	14.3	24	85.7
Duration of symptoms								
1-7 d	51	47.2	57	52.8	11	16.2	57	83.8
>7 d	15	27.8	39	72.2	11	22.0	39	78.0
Missing	2		3				3	
>1 partner in the previous 3 mo								
Yes	18	51.4	17	48.6	5	22.7	17	77.3
No	50	37.9	82	62.1	17	17.2	82	82.8
Commercial sex in the previous 3 mo								
Yes	13	43.3	17	56.7	6	17.6	28	82.3
No	55	40.1	82	59.9	16	19.1	68	80.9

	HSV						<i>T pallidum</i>					
	Positive			Negative			Positive			Negative		
	n	%	n	%	n	%	n	%	n	%	n	%
Condom use last sex main partner												
Yes	16	36.4	28	63.6			9	21.9	32	78.1		
No	49	42.6	66	57.4			10	14.5	59	85.5		
Not applicable	3		5				3		8			
Condom use last sex casual partner												
Yes	32	50.0	32	50.0			1	5.6	17	94.4		
No	35	37.2	59	62.8			21	20.4	82	79.6		
Not applicable	1		8									
Perceived HIV status												
Positive	34	54.0	29	46.0			11	27.5	29	72.5		
Negative	16	41.0	23	59.0			4	14.8	23	85.2		
Unknown	18	27.7	47	72.3			7	13.0	47	87.0		
Actual HIV status												
Positive	41	53.9	35	46.1			9	20.5	35	79.5		
Negative	21	28.4	53	71.6			11	17.2	53	82.8		
Missing	6		11				2		11			
Gonorrhea NAAT												
Positive	8	29.6	19	70.4			8	29.6	19	70.4		
Negative	60	42.9	80	57.1			14	14.9	80	85.1		
Chlamydia NAAT												
Positive	3	15.0	17	85.0			4	19.1	17	80.9		
Negative	65	44.2	82	55.8			18	18.0	82	82.0		

* HSV analysis limited to those negative for *T pallidum* (n = 167); *T pallidum* analysis limited to those negative for HSV (n = 121).

TABLE 4
 Treponemal and Nontreponemal Test Results Among Patients With Genital Ulcer Disease in Zimbabwe

	M-PCR Results for <i>T. pallidum</i>			
	Positive	%	Negative	%
RPR				
n Tested	29		152	181
Positive	19	65.5	18	11.8
Negative	10	34.5	134	88.2
TPHA				
n Tested	27		138	165
Positive	21	77.8	21	15.2
Negative	6	22.2	117	84.8
RPR/TPHA				
n Tested	27		138	165
Both positive	15	55.6	11	8.0
Both negative	2	7.4	110	79.7
Either positive	25	92.6	28	20.3
			53	32.1

TABLE 5

Urogenital Chlamydia and Gonorrhea Infections

	Chlamydia		Gonorrhea		Chlamydia or Gonorrhea	
	n	%	n	%	n	%
Women						
n= 100	16	16.0	23	23.0	31	31.0
Vaginal discharge						
Observed (n = 30)	6	20.0	6	20.0	10	33.3
Not observed (n = 68)	9	13.2	16	23.5	20	29.4
Missing (n = 2)						
Men						
n= 98	11	11.2	18	18.4	23	23.5
Urethral discharge						
Observed (n = 15)	1	6.7	6	40.0	6	40.0
Not observed (n = 71)	8	11.3	10	14.1	14	19.7
Missing (n = 12)						
All						
n= 198	27	13.6	41	20.7	54	27.3
Discharge						
Observed (n = 45)	7	15.6	12	26.7	16	35.6
Not observed (n = 139)	17	12.2	26	18.7	34	24.5
Missing (n = 14)						